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Germline Specification: Small Things Have a Big Role

Germline cell fate is specified by localized OSK, VAS and other components in the pole plasm of the *Drosophila* embryo. New work shows that a PIWI-mediated miRNA pathway contributes to this process by regulating OSK and VAS localization.

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In many species, germline cells are separated from somatic cells during early embryogenesis. Studies in *Drosophila* have revealed that germline cells form by incorporation of pole plasm, which is assembled in the posterior pole of the oocyte during oogenesis. The pole plasm contains many RNAs and maternal proteins including Oskar (OSK), Vasa (VAS) and Tudor (TUD) [1] (Figure 1). OSK accumulation in the pole plasm requires functional VAS and TUD, which interact with the translation initiation factor eIF5B and mitochondrial rRNAs, respectively [2–4]. Thus, these proteins specify germline cell fate in the early fly embryo presumably by regulating translation and/or mRNA localization, eventually giving rise to 20–30 primordial germ cells (PGCs), also known as pole cells [1]. This mode of germline specification is conserved across many different species; however, little is known regarding the molecular machinery responsible for this process.

PIWI is essential for maintaining germline stem cells in adult *Drosophila* ovaries and testes [5]. PIWI is a member of the conserved PIWI/Argonaute (AGO) family of proteins containing PAZ and PIWI domains that bear RNA-binding activity. Additionally, this family includes AGO-1, AGO-2 and Aubergine (AUB). AGO-1 and AGO-2 are required for producing the 21–23 nucleotide long regulatory RNAs that are used by the siRNA and miRNA pathways [6,7], respectively. PIWI and AUB are essential for generating the 29–30 nucleotide long rasiRNAs that maintain genome stability in the *Drosophila* germline [8]. In a recent paper in *Current Biology*, Megosh and colleagues [9] have provided compelling evidence that a PIWI-mediated miRNA pathway plays a key role in specifying germline cell fate in *Drosophila* through the regulation of OSK and VAS protein localization.

In search for potential PIWI functions during embryogenesis, Megosh *et al.* [9] have discovered that, like OSK, VAS and TUD, PIWI is specifically localized to the pole plasm and pole cells. Similar to removal of maternal OSK, VAS or TUD, depletion of maternal PIWI results in fewer pole cells, which is likely to be caused by reduced accumulation of OSK, VAS and TUD at the posterior pole. Conversely, increased maternal PIWI dosage results in an increased number of pole cells, presumably due to higher levels of OSK, which can lead to more pole cells [10]. Although both PIWI and OSK control pole cell production in a dosage-dependent manner, there are significant differences between the two genes. Unlike PIWI, OSK is sufficient to induce ectopic pole cells, even when it is ectopically localized to the anterior pole and is essential for posterior embryonic patterning. PIWI is neither sufficient for pole cell formation, nor required for posterior patterning. Interestingly, PIWI is required for the correct localization of OSK and VAS proteins but not for their expression, and thus, upregulation of OSK/VAS caused by increased PIWI dosage is likely due to their increased posterior localization. The increased posterior localization could be mediated by physical interactions between PIWI and VAS, as VAS is required for posterior OSK localization [2]. It is of great interest to reveal the mechanism of how an increased amount of PIWI protein can cause accumulation of more OSK protein and whether OSK mediates the pole cell increase caused by high PIWI dosage.

The study by Megosh *et al.* [9] provides critical insight into how PIWI might control germ cell specification (Figure 1). The first hint comes from the biochemical evidence that PIWI forms protein complexes with DCR-1 and dFMRP, two essential components of the miRNA pathway, but not with the RNAi pathway component DCR-2. Removal of maternal DCR-1 and dFMRP — but not DCR-2 — also results in fewer pole cells, but has no effect on posterior patterning like depletion of maternal PIWI. This suggests that PIWI is likely to function in the miRNA pathway to regulate pole cell production. However, the study by Megosh *et al.* [9]

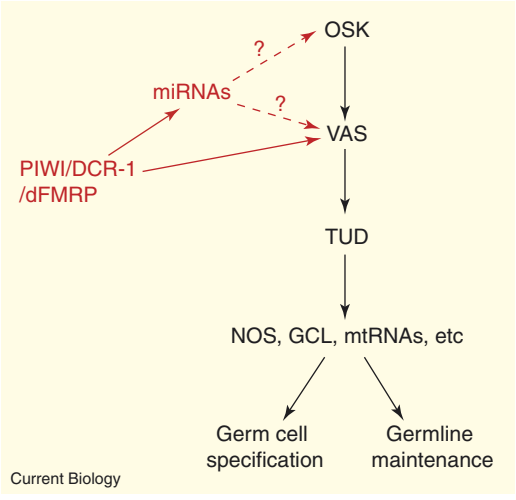

Figure 1. Revised model for germ cell specification in *Drosophila*. The components in black and their functional sequence have been previously established [4], while the components in red are identified in the study by Megosh *et al.* [9]. The PIWI/DCR-1/dFMRP-mediated miRNA pathway can either directly or indirectly modulate the localization of OSK and VAS in the *Drosophila* embryo.

does not address whether PIWI/DCR-1/dFMRP control pole cell formation, proliferation or survival. One recent study by Deshpande *et al.* [11] sheds some light on how dFMRP at least controls pole cell formation and also potentially regulates pole cell stability. The maternal dFMRP depleted embryos display defects in arrangements and concentrations of Anillin in polar buds, resulting in formation of fewer polar cells [11]. This is an important finding because Anillin is a protein known to be essential for cytokinesis. Interestingly, as *nanos* and *germ cell less* mutant pole cells do, the *dfmrp* mutant pole cells also exhibit defects in transcriptional silencing, which is required for maintaining germ cell fate. Thus, it is important to know if *piwi* and *dcr-1* mutant pole cells behave similarly in transcriptional silencing. If so, the miRNA pathway is required not only for pole cell formation but also for germ cell fate maintenance.

Even if PIWI/DCR-1/dFMRP are required for pole cell formation and possibly germ cell fate maintenance, it remains unclear how these proteins control these processes mechanistically. There are several possible scenarios (Figure 1). First, these proteins might directly interact with pole cell determinants for their posterior localization. In support of this idea, PIWI is shown by Megosh *et al.* [9] to be directly associated with VAS. Second, PIWI/DCR-1/dFMRP might control posterior localization of VAS/OSK/TUD indirectly through miRNAs as these proteins have been shown to be involved in miRNA production/function [12]. Consistent with this idea, a miRNA, miR-6, has been shown to be essential for pole cell formation [13]. It would be extremely interesting to investigate if depletion of miR-6 causes any defects in posterior VAS/OSK/TUD localization and in transcriptional silencing of pole cells. Lastly, PIWI/DCR-1/dFMRP might regulate pole cell formation through a combination of direct and indirect routes. In contrast with the lack of pole cells observed in OSK/TUD depleted embryos, depletion of PIWI/DCR-1/dFMRP-1 only results in a partial reduction of pole cells, suggesting that there might be a second pathway that is redundant with the PIWI-mediated miRNA pathway or that the miRNA pathway only plays a regulatory but not determining role in pole cell formation. Interestingly, maternal depletion of AUB [14] and AGO-2 [11] has a similar effect on pole cell formation, supporting the existence of a pathway redundant to the PIWI-mediated pathway. In the future, in order to gain more mechanistic insight into how PIWI and the miRNA pathway control pole cell formation, these different possibilities need to be addressed experimentally.

Recent studies on PIWI and its murine homolog have made things even more complicated [15]. MIWI, a murine homolog of PIWI, binds to a new class of 29–30 nt small RNAs, named piRNAs, in the mouse testis, and production of rasiRNA in *Drosophila* requires AUB and PIWI but not DCR-1 and DCR-2. An immediate question is whether the pi/rasiRNA pathway is also involved in regulation of pole cell formation and stability. This question could be effectively answered by examining pole cell formation in the PIWI/DCR-1 double maternally depleted embryos. Furthermore, PIWI has been shown to be required in niche cells for controlling germline stem cell maintenance in the adult ovary [16], while PIWI and DCR-1 are required in germline stem cells for controlling their division rate [16,17]. The study by Megosh *et al.* [9] also raises the interesting possibility that miRNAs produced by PIWI could be important for regulating niche signaling activity and germline stem cell mitotic activity. Whatever the future holds for us, further investigations will greatly enhance our understanding of the PIWI-mediated miRNA pathway and its relationship with germline fate, revealing the secrets of the continuity of life.

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